# Supporting Information

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### SI Materials and Methods

**Immunoblotting, Co-IP and Statistical Analysis.** Immunoblotting using ~80 $\mu$g of protein lysate from each testis sample prepared with an IP lysis buffer (see text) was performed as described (1). Co-IP was performed as described (1) using ~700 $\mu$g of protein of testis lysates from each sample. Each experiment was repeated at least three or four times. All samples within an experimental set were processed simultaneously to minimize inter-experimental variations. Germ cells (2) and seminiferous tubules were isolated from adult rat testes with negligible contamination of other somatic cells (3) as detailed elsewhere from this laboratory. All experiments reported herein were repeated at least three to four times by using different batches of Sertoli cells excluding pilot experiments which were used to establish the optimal experimental conditions. For immunohistochemistry and fluorescent microscopy studies, micrographs reported here are the results of representative sets of experiments. For statistical analysis, data from treatment groups were compared to the corresponding controls by ANOVA followed by Tukey's Honest Significant Different test using GBSTAT (version 7) (Dynamic Microsystems).

**General Methods.** Immunoblotting, Co-IP, an d statistical analysis were performed as described in SI *Materials and Methods*.


1. Yan HHN, Cheng CY (2005) Blood-testis barrier dynamics are regulated by an engagement/disengagement mechanism between tight and adherens junctions via peripheral adaptors. *Proc Natl Acad Sci USA* 102:11722–11727.
2. Aravindan GR, Pineau C, Bardin CW, Cheng CY (1996) Ability of trypsin in mimicking germ cell factors that affect Sertoli cell secretory function. *J Cell Physiol* 168:123–133.
3. Lee NPY, Mruk DD, Lee WM, Cheng CY (2003) Is the cadherin/catenin complex a functional unit of cell-cell actin-based adherens junctions in the rat testis? *Biol Reprod* 68:489–508.

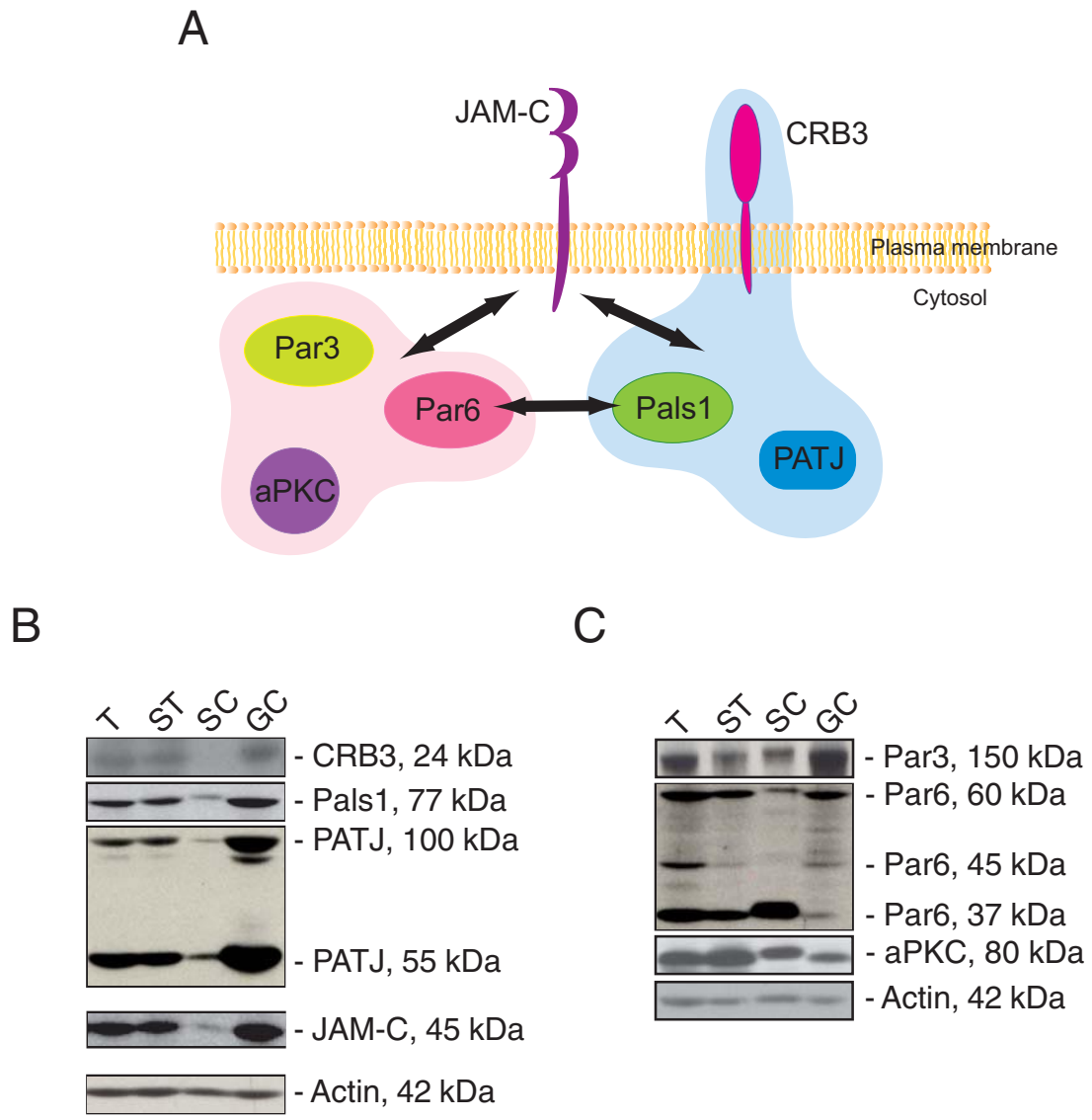


**Fig. S1.** Expression and cellular distribution of polarity proteins in rat testes. (*A*) Molecular interactions between the two known polarity complexes, namely the CRB complex, which is composed of CRB3, Pals1, PATJ, and the Par complex, which is constituted by Par3, Par6 and aPKC and their interactions with JAM-C (arrows). In addition, these two complexes are physically and functionally connected via Pals1 and Par6 interaction (arrow). (*B*) Testis (T), seminiferous tubule (ST), Sertoli cell (SC), and germ cell (GC) lysates (≈80 $\mu$g protein per lane) were used for immunoblot analysis with different antibodies (see Table S1), illustrating the presence of CRB3, Pals1, PATJ, and JAM-C in rat testes. Membranes were stripped and reprobed for actin to assess equal protein loading. (*C*) Components of the Par polarity complex, namely Par3, Par6, and aPKC, were also detected in testes and seminiferous tubules, Sertoli, and germ cells isolated from rat testes. Data shown are representative results from three different experiments that yielded similar results. JAM-C, and component proteins of the CRB and Par polarity complexes were found in Sertoli and germ cells. However, the steady-state protein level of CRB3 in Sertoli cells was virtually undetectable (see *B*), but the interacting partners of CRB3, such as Pals1 and PATJ, were detected in Sertoli cells even though their levels were considerably lower than germ cells, illustrating that they could be associated with another integral membrane protein other than CRB3 in Sertoli cells (see *B*). The apparent $M_r$ of the target proteins shown in *B* and *C* are consistent with earlier reports as indicated by the corresponding vendors (Table S1). The predominant isoforms of PATJ in the testis were at 100 and 55 kDa, consistent with those observed in brain and kidney lysates as indicated by the manufacturer (see Table S1), instead of ≈180–200 kDa found in tumor cell lines such as Caco-2 or HeLa cells (1). The Par3 antibody specifically recognized the 150-kDa isoform in testis and cell lysates. For Par6, three isoforms of Par6$\alpha$, Par6$\beta$, and Par6$\gamma$, as indicated by the manufacturer (see Table S1), were detected with the 37- and 45-kDa bands corresponded to Par6$\alpha$, whereas the 60-kDa band corresponded to Par6$\beta$ and Par6$\gamma$, which share similar $M_r$. Testes were obtained from adult rats at 90 days of age, and seminiferous tubules and germ cells were isolated from testes of adult rats at 90 days of age as described (2, 3), and Sertoli cells were isolated from 20-day-old rat testes as described (4).


1. Lemmers C, *et al.* (2002) hINADl/PATJ, a homolog of Discs Lost, interacts with Crumbs and localizes to tight junctions in human epithelial cells. *J Biol Chem* 277:25408–25415.
2. Zwain IH, Cheng CY (1994) Rat seminiferous tubular culture medium contains a biological factor that inhibits Leydig cell steroidogenesis: Its purification and mechanism of action. *Mol Cell Endocrinol* 104:213–227.
3. Aravindan GR, Pineau C, Bardin CW, Cheng CY (1996) Ability of trypsin in mimicking germ cell factors that affect Sertoli cell secretory function. *J Cell Physiol* 168:123–133.
4. Cheng CY, Mather JP, Byer AL, Bardin CW (1986) Identification of hormonally responsive proteins in primary Sertoli cell culture medium by anion-exchange high-performance liquid chromatography. *Endocrinology* 119:1914–1922.

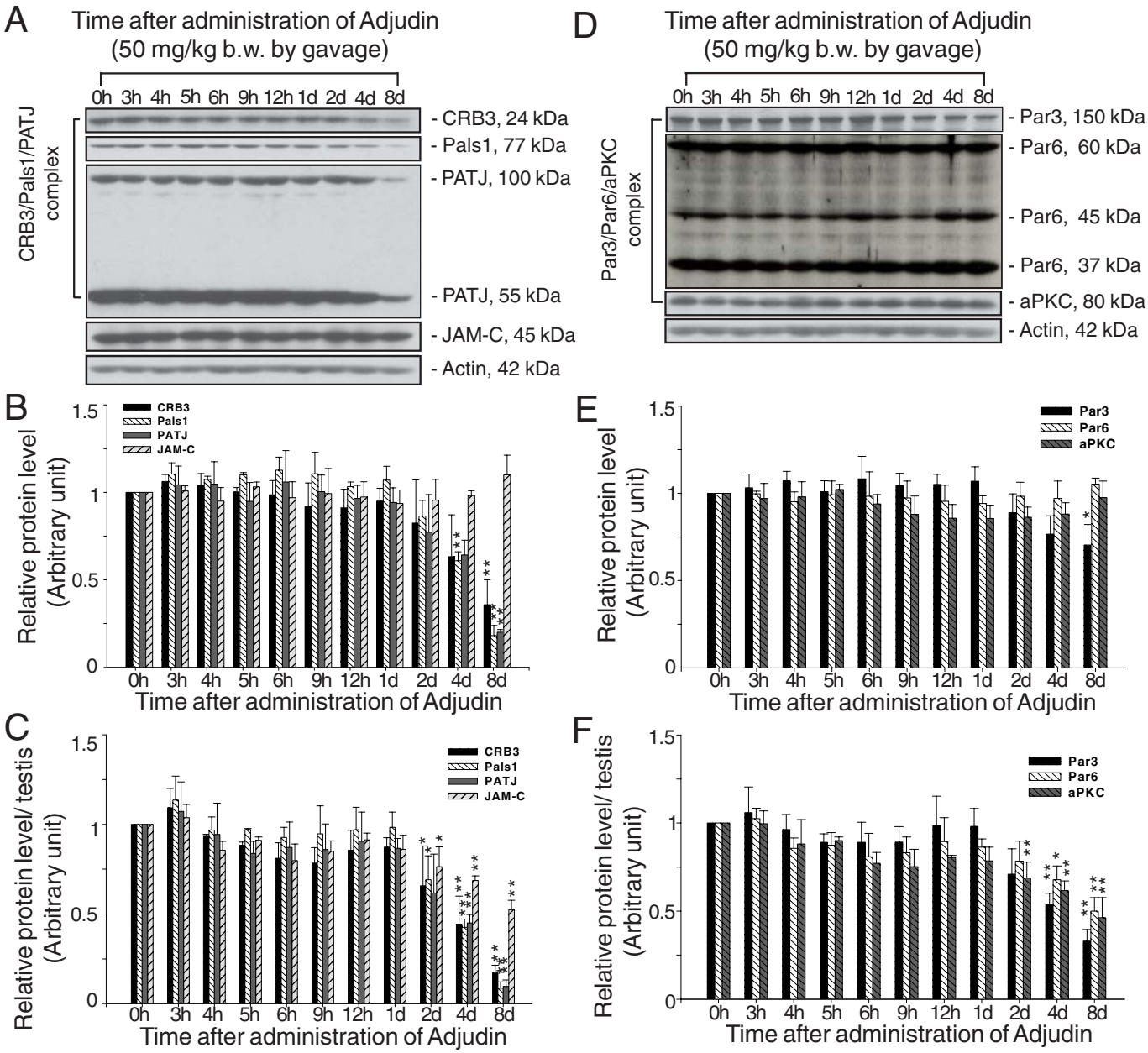


**Fig. S2.** Changes in the steady-state protein levels of polarity proteins in the testis during Adjudin-induced germ cell loss from the seminiferous epithelium. (*A*) Rats (≈300 gm b.w.) were treated with 50 mg/kg body weight of Adjudin by gavage and euthanized by $CO_2$ asphyxiation at specified time points. Testis lysates (≈80 $\mu$g protein per lane) by were resolved by SDS/PAGE, and blots were probed with specific antibodies against CRB3, Pals1, PATJ, and JAM-C. Protein concentration in lysates was estimated by using the DC protein assay kit (Bio-Rad). Same blots were reprobed with actin to serve as protein loading controls. Relative protein levels at 0 h were arbitrarily set at 1. (*B*) Significant decrease in relative steady-state protein levels in the CRB3/Pals1/PATJ complex was detected starting at 8-day (d) posttreatment but not JAM-C as shown in the histogram after densitometric scanning of immunoblots, such as the one shown in *A*. Protein levels from three independent experiments were determined, and each bar is the mean ± SD. (*C*) Relative protein levels shown in *B* were normalized against changes in testis weight after Adjudin treatment as a result of germ cell loss from the seminiferous epithelium. Significant decrease in relative protein levels of CRB3, Pals1, PATJ, and JAM-C was detected starting from 4 days. (*D–F*) The same procedures were performed as in *A–C* to study the changes in protein levels of the Par3/Par6/aPKC complex after Adjudin treatment. (*E*) No significant difference in steady-state protein levels was detected for Par6 and aPKC, except a mild but significant drop in Par3 protein level was detected at 8 days. (*F*) Significant declines in Par3, Par6, and aPKC protein levels were found after normalization of the loss in testis weight starting from 4 days. *, $P < 0.05$; **, $P < 0.01$ by one-way ANOVA. A significant decrease in protein levels of CRB3, Pals1, and PATJ was detected only by day 8 post-Adjudin treatment (see *A* and *B*), probably because the loss of germ cells from the epithelium as germ cells contributed significantly to the CRB protein pool in the testis (see Fig. 1*B*). No significant drop in JAM-C was observed up to 8 days (see *A* and *B*), which is unusual because JAM-C is predominantly expressed by germ cells (Fig. 1*B*). This suggests that JAM-C might have been actively produced by Sertoli cells at the time, illustrating a surge in its Sertoli cell expression. After Adjudin treatment, there was a gradual, but significant, loss in testis weight as germ cells were depleting from the testis, altering the cellular composition in the seminiferous epithelium. We thus estimated the organ content of different target proteins for comparison. We found that the relative protein levels of CRB3, Pals1, PATJ, and JAM-C significantly reduced from 4 days (see *C*). Immunoblot analysis was also performed to examine any changes in protein levels of the Par complex. No significant change in protein levels was observed for Par6 and aPKC except a mild decrease in Par3 (150-kDa isoform) at 8 days (see *D* and *E*). However, when the relative protein levels were normalized against the decrease in testis weight, a significant decline was observed for Par3, Par6, and aPKC only at 4 and 8 days (see *F*).

**Table S1. Antibodies used for different experiments**

| Antibody (host animal) | Vendor | Catalog no. | Application/working dilution |
|---|---|---|---|
| Goat anti-CRB3 | Santa Cruz Biotechnology | sc-27906 | WB (1:200) |
| Rabbit anti-Pals1 | Upstate Biotechnology | 07–708 | WB (1:1,000) |
| Goat anti-PATJ | Abcam | ab8225 | WB (1:500) |
| Rabbit anti-JAM-C | Zymed Laboratories | 40–9000 | WB (1:150) |
| Goat anti-actin | Santa Cruz Biotechnology | sc-1616 | WB (1:200) |
| Rabbit anti-Par3 | Zymed Laboratories | 36–2301 | WB (1:1,000) |
| Rabbit anti-Par3 | Upstate Biotechnology | 07–330 | WB (1:500) |
| Rabbit anti-Par6 | Abcam | ab45394 | WB (1:1,000) |
| | | | IHC (1:100) |
| | | | IF (1:75) |
| | | | IP |
| Rabbit anti-aPKC, recognizes PKCλ and PKCι | Santa Cruz Biotechnology | sc-216 | WB (1:200) |
| Goat anti-nectin-3 | Santa Cruz Biotechnology | sc-14806 | IF (1:25) |
| Mouse anti-occludin | Zymed Laboratories | 33–1500 | IF (1:50) |
| Mouse anti-N-cadherin | Zymed Laboratories | 33–3900 | IF (1:75) |
| Mouse anti-γ-catenin | BD Transduction Laboratories | 610254 | WB (1:1,000) |
| | | | IF (1:75) |
| Goat anti-JAM-C | Santa Cruz Biotechnology | sc-23005 | IP |
| Rabbit anti-Src kinase | Upstate Biotechnology | 05–772 | IP |
| Rabbit anti-occludin | Zymed Laboratories | 71–1500 | WB (1:150) |
| Rabbit anti-JAM-A | Zymed Laboratories | 36–1700 | WB (1:300) |
| | | | IF (1:50) |
| Rabbit anti-ZO-1 | Zymed Laboratories | 61–7300 | WB (1:300) |
| | | | IF (1:50) |
| Rabbit anti-N-cadherin | Santa Cruz Biotechnology | sc-7939 | WB (1:200) |
| Rabbit anti-α-catenin | Santa Cruz Biotechnology | sc-7894 | WB (1:200) |
| | | | IF (1:75) |
| Rabbit anti-β-catenin | Santa Cruz Biotechnology | sc-7199 | WB (1:200) |
| Goat anti-nectin-2 | R & D Systems | AF2229 | IF (1:50) |

WB, immunoblotting; IHC, immunohistochemistry; IF, immunofluorescence microscopy; IP, immunoprecipitation. Antibodies listed are those that yielded satisfactory results and were used in experiments reported herein and each of the antibodies cross-reacted with the corresponding rat protein as indicated by the manufacturer. Antibodies raised in goat and rabbit are polyclonal antibodies, those raised in mouse are monoclonal antibodies. It must be noted for most antibodies, such as Par3, Par6, JAM-C, nectin-2, JAM-A, and occludin, as many as three to four different vendors were used for initial pilot experiments. HRP-conjugated secondary antibodies for immunoblotting were obtained from Santa Cruz Biotechnology. For immunofluorescence microscopy, FITC goat anti-rabbit and anti-mouse IgG conjugate or Cy3 goat anti-rabbit IgG conjugate were from Zymed Laboratories. FITC-conjugated donkey anti-goat IgG was purchased from Chemicon International. Among the antibodies against different components of the two polarity complexes that were used for immunoblot analysis and shown in this report and Figs. S1 and S2, we also used these antibodies for IHC with frozen or paraffin sections, and from different vendors, some of which are not listed, only the anti-Par6 antibody from Abcam yielded consistent staining results, which was used for our experiments.